Research Article

Premarital hemoglobinopathy screening in Kocaeli, Turkey: a crowded industrial center on the north coast of Marmara Sea

Marmara denizinin kuzey kıyısında kalabalık bir endüstri merkezi olan Kocaeli'de evlilik öncesi hemoglobinopati taraması

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Abstract

Objective: Premarital hemoglobinopathy screening is one of the important procedures of hemoglobinopathy control programs. This is the first report about the prevalence of hemoglobinopathies in Kocaeli.

Materials and Methods: The study covered screening from July 2005 to the end of December 2008. Under the auspices of the Ministry of Health and regional health authorities, blood samples of the couples were obtained during admission to the marriage office. Complete blood counts and hemoglobin variant analysis were performed with automatic counter and high pressure liquid chromatography technique. Genetic counseling was given to carriers of thalassemia and abnormal hemoglobins.

Results: A total of 88,888 people were screened. The frequencies of β-thalassemia trait and sickle cell anemia trait were 0.89% and 0.05%, respectively. The frequency of couples with high-risk of having a sibling with homozygous hemoglobinopathy was 0.01%.

Conclusion: The prevalence of β-thalassemia trait and sickle cell anemia trait was quite low and reflects the frequency in eastern and northern Anatolia and migration to Kocaeli from these geographic regions. Although frequency is low, the chronic transfusion requirement, high cost of chelating, organ damage, painful crisis and other crisis, and availability of stem cell transplantation only for a limited number of patients with compatible sibling donors justify premarital screening studies even in regions with lower prevalence such as Kocaeli. *(Turk J Hematol 2009; 26: 62-6)*

Key words: β-thalassemia trait, premarital screening, sickle cell anemia trait.

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Özet

Amaç: Evlilik öncesi hemoglobinopati taraması hemoglobinopati kontrol programının önemli bir parçasıdır. Bu çalışma Kocaeli'deki hemoglobinopati sıklığına ilişkin ilk rapordur.

Yöntemler: Çalışma Haziran 2005-Aralık 2008 dönemini kapsamaktadır. Sağlık Bakanlığının ve İl Sağlık Müdürlüğünün hemoglobinopati kontrol programı kapsamında evlenecek çiftlerin kanları nikah işlemleri için başvuruları sırasında alınmıştır. Tam kan sayımları ve hemoglobin varyant analizleri otomatik sayıcı ve yüksek basınçlı sıvı kromotografisi tekniği ile çalışılmıştır. Talasemi ve anormal hemoglobin taşıyıcılarına genetik danışma verilmiştir.

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Bulgular: Toplam 88888 kişi taranmıştır. β-talasemi taşıyıcılığı sıklığı % 0,89, orak hücreli anemi taşıyıcılığı sıklığı % 0.05, homozigot hemoglobinopatili çocuğa sahip olma olasılığı olan çift oranı %0,01 bulunmuştur
Sonuç: Kocaeli ilinde β-talasemi ve orak hücreli anemi taşıyıcılığı sıklığı oldukça düşüktür. Sıklık kuzey ve doğu Anadoludaki oranları ve Kocaeli'ye bu bölgelerden olan göçü yansıtmaktadır. Bu hastalıklardaki kronik transfüzyon gereksinimi, şelazyonun yüksek maliyeti, organ hasarları, ağrılı krizler ve diğer krizler, kök hücre naklinin ancak dokusu uyumlu kardeşi olan sınırlı sayıda hastaya yapılabilmesi, evlilik öncesi tarama çalışmalarının Kocaeli gibi taşıyıcılık oranlarının düşük olduğu bölgelerde de gerekli olduğunu düşündürmektedir. *(Turk J Hematol 2009; 26: 62-6)*
Anahtar kelimeler: β,-talasemi taşıyıcılığı, evlilik öncesi tarama, orak hücreli anemi taşıyıcılığı

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Introduction

Thalassemias are the most common single-gene disorders in the world and are real health problem, especially in the Mediterranean basin and in parts of Asia and the Middle East.

The expected number of carriers is 1,400,000 and the number of patients is approximately 5,000. In 2006, the Ministry of Health and Turkish National Hemoglobinopathy Council reported the results of screening studies (377,339 healthy subjects) from 16 different cities and recorded the average frequency of β-thalassemia trait as 4.3%. The highest prevalence of β-thalassemia trait (13.1%) was in Antalya and Çukurova regions (10%) [1].

Kocaeli is a crowded city of Turkey on the north coast of Marmara sea with a population of approximately 1,438,000 and 28,400 births in 2008. As an important center of industry and trade, its population is progressively increasing mainly due to migration. Turkey's population is a mosaic of various ethnicities. This feature is reflected in the distribution of hemoglobinopathies, thalassemia carriage being the most prevalent, followed by sickle cell hemoglobinopathy and others. Although 700 structural hemoglobin variants have been identified, only three (HbS, HbC and HbE) reach high frequencies in the world [2].

Thalassemia major and sickle cell anemia are real burdens to the individual and family and health budgets. Prevention of births of affected children must be the main national strategy. The Ministry of Health and the Turkish National Hemoglobinopathy Council initiated a national hemoglobinopathy control program in 33 provinces, including Kocaeli [1].

The Kocaeli Hemoglobinopathy Screening Center (HSC) started premarital screening in July 2005. In the present report, the results of a 3.5-year premarital screening program are presented as the first report from Kocaeli covering an extensive number of people.

Material and Methods

The premarital hemoglobinopathy screening program is operated under the auspices of the Ministry of Health and regional health authorities in Kocaeli. This study covered the results of screening from July 2005 to the end of December 2008. Ethical approval of Kocaeli Health Directorate was obtained for reporting the results. During admission to the marriage office, all couples were referred to local primary health care centers to give venous blood samples to screen for some contagious diseases and hemoglobinopathies. These tests were mandatory. The Provincial Directorate of Health conducted the logistic organization, and blood samples collected in primary health care centers of all districts (İzmit, Derince, Gebze, Körfez, Gölcük, Kandıra, Karamürsel) were delivered to Kocaeli HSC weekly in cold chain.

In the HSC, complete blood counts and hemoglobin variant analysis of both partners were performed with automatic counter and high pressure liquid chromatography technique.

Instruments used were: Mindray Medical Instruments Ltd. BC 5500 blood counter; Agilent Technologies Inc. 1200 series hemoglobin variant analyzer, distributor Betamed Tibbi Malzeme Ltd. (used in 2005-2007); Primus Corporation, Ultra 2 hemoglobin variant analyzer, distributor Biocan Tıp Laboratuvar ve Tıbbi Malzemeler Ltd. (2008). The equipment performed hemoglobin variant analysis by cation exchange chromatography in conjunction with gradient elution from hemolyzed EDTA whole blood. The separated hemoglobin fractions were monitored by means of absorption, and the chromatogram obtained was recorded and stored by the internal computer. To avoid technical errors, quality control was performed daily with commercially available control samples.

The subjects were considered to have the β-thalassemia trait if they had a mean corpuscular volume (MCV) <80 fL and/or a mean corpuscular hemoglobin (MCH) level of <27 pg and a hemoglobin (Hb)A2 level of >3.5% or a HbF level of >2%. HbF, S, C, D, E and other variants were also detected. HbA2 level between 3.1 and 3.5 was considered borderline. After evaluation of chromatograms by the physicians of the Center (J. Bayram and V. Şenkal), carrier couples were invited to the HSC for genetic counseling. High-risk couples were informed about possible deleterious consequences on their offspring and about prenatal diagnosis and medical abortion of an affected fetus. They were informed that the couple took full personal responsibility in proceeding with their marriage. Cases with presumed iron deficiency or with uncertain results were referred to Kocaeli University Hematology outpatient unit or the Genetics Department of Kocaeli University for further analysis (strip assays for mutation analysis of alpha- and beta-thalassemia). All demographic data of the couples (age, place of residence, place of birth and contact numbers) and detailed results of the assays were recorded in a computer prospectively.

During counseling, complete blood counts were repeated in fresh blood samples to avoid any difference in red blood cell indices due to storage.

The frequency of hemoglobinopathy variants was calculated.

Results

In the last 3.5 years, 88,888 people were screened in the premarital screening program. The frequencies of β-thalassemia trait, sickle cell trait, HbD trait and HbC trait were 0.89%, 0.05%, 0.09% and 0.009%, respectively. The frequency of high-risk couples was 0.01% (Table 1). Other variants are presented in Table 2.

There were borderline HbA2 levels in 9 samples and isolated high HbF levels with normal hematologic values in 78 samples.

Discussion

In the present study, prevalence of β-thalassemia carriers was 0.89%, which is not very high. When compared to some screening studies from Turkey, prevalence of the thalassemia trait in Kocaeli is similar to that of Erzurum. Sickle cell carriage, which was 0.05%, was similar to that of Konya (Table 3) [3-9]. Çavdar O.A et al performed the first screening study in Turkey by measuring HbA2 levels in Ankara from 900 blood samples of Turkish Army members and Medical School and 100 cord blood samples collected from the City Maternity Hospital and reported an incidence of 1.66% for β-thalassemia trait [10]. The highest β-thalassemia prevalence was in Antalya and Thrace (10%) and Muğla (4.8%). The frequency is lowest in eastern and northern Anatolia [11]. In a screening study from schools of Gaziantep, the prevalence of β-thalassemia trait was 1.84%, while it was 1% (n=995) in the Elbistan district of Kahramanmaraş and 3% in İzmir [12-14]. Kocaeli as a region of industry has migration from north, east and central Anatolia. The low prevalence may be a reflection of this heterogeneous population. National population statistics (data of Türkiye İstatistik Kurumu) showed that between 1985-1995, 83,262 people migrated to Kocaeli, with a migration rate of 108.2 per thousand. This migration rate was even higher than in İstanbul, which was 107 per thousand during the same period. In 2000, the year following the Marmara earthquake, the increase in the population in Kocaeli was only 211 people because there was also migration from Kocaeli.

The prevalence of sickle cell trait was also low in the present study. Sickle cell trait is the second most common hemoglobinopathy in Turkey, with the highest frequency in the Çukurova region (Adana, Hatay and Mersin, 10%) [15].

In the present study, HbD, HbE Saskatoon, HbO Arab and HbC seemed to be the most prevalent variants after β-thalassemia and sickle cell hemoglobinopathy. In a screening study from Kahramanmaraş, the prevalences of HbD and HbO Arab carriers were 0.28% and 0.013%, respectively [16]. Heterozygous states of Hb C, D, and E occur globally with sufficient frequency to be considered a polymorphism [17]. HbE is the most common structural hemoglobin variant globally; it is innocuous in heterozygous and homozygous state but HbEβ thalassemia is a serious condition [17]. Detection of some variants depends on the equipment. Although the detected variants are presented in Table 3, we believe that it is not possible to give a true prevalence. Many of the variants were detected with an analyzer we used in 2008. A genetic analysis to confirm these variants may be necessary.

In premarital screening studies it is very important to inform couples who are going to marry and each of them are carriers have the risk to have homozygote child with thalassemia.

In a previous study, premarital screening was performed on 2113 couples and both partners were found to be carriers in 35 families. Prenatal diagnosis was performed in 10 pregnancies of these families [18] instead of 19. Following studies were done after this first premarital screening study [4,7,9,18,19].

In the screening study, there were 9 people with borderline HbA2 and 78 people with elevated HbF. Silent carriers of β-thalassemia and the possibility of other thalassemic types such as α-thalassemia carriers represent a significant dilemma in defining disorders. The presence of a single β-thalassemia allele is usually associated with hypochromic microcytic red cells and increase in HbA2 levels, but some alleles may show a silent phenotype (type 1) with normal or borderline hematological levels and HbA2 due to β-thalassemia genotype or the interaction of this genotype with other molecular defects (α-, δ-thalassemia). People with borderline HbA2 (3.1-3.9) must undergo molecular genetic studies, particularly if the partner is a carrier of β-thalassemia, to avoid missing at-risk couples [20]. A typical hematological feature of the β-thalassemia trait is MCV <79 fl, MCH <27 pg, and HbA2 >3.4. In β-thalassemia trait, the level of HbF is elevated in 50% of cases, usually to 1 to 3% and rarely to 5% [17]. If there is a combination of β- and

Table 1. Screened population and frequency of hemoglobinopathies during premarital screening

Year	Screened population	β-thalassemia carrier	β-thalassemia intermedia	Hb S carrier	Borderline HbA2 values	High HbF >2%**	High-risk couples
2005*	9,495	67	0	6	6	17	2
2006	25,019	199	0	6	2	20	3
2007	27,310	262	1	21	0	21	4
2008	27,064	265	0	17	1	20	3
total	88,888	793	1	50	9	78	12

*6 months of 2005.

** Isolated high HbF with normal hematologic values and normal HbA2

δ-thalassemia, the clinical picture may resemble the β-thalassemia trait. HbF level is higher, in the range of 5-20%, and the A2 level is normal or slightly reduced. MCV and MCH may be higher [17]. In α-thalassemia trait, Hb electrophoresis is normal. There may be minimal hematologic changes and there is no reliable way of making the diagnosis except by DNA analysis.

As is known people homozygotes for (δβ)° thalassemia have 100% HbF and their blood shows mild thalassemic changes with reduced MCV and MCH values, very similar to the β-thalassemia trait. Heterozygotes have approximately 20-30% HbF with slightly reduced HbA2 values and completely normal blood pictures [17].

Table 2. Some hemoglobin variants detected during screening

	2005	2006	2007*	2008	Total
Hb D	12	41	37	33	87
Hb E Saskatoon	2	1	2	10	15
Hb O Arab	-	-	-	13	13
Hb C	2	4	5	1	8
B Wayne	-	-	-	5	5
Hb G-Coushatta	-	-	-	2	2
Hb G-Copenhagen	-	-	-	2	2
HbS Florida	-	-	-	2	2
Hb Tayne	-	-	-	1	1
Hb Riyadh	-	-	-	1	1
Hb Strumica	-	-	-	1	1
Hb Le-Lamentin	-	-	-	1	1
Hb Hasharon	-	-	-	1	1
Hb Loves Park	-	-	-	1	1
Hb Rhode Island	-	-	-	1	1
Hb J-Anatoliae	-	-	-	1	1
Hb J-Meerut	-	-	-	1	1
Hb Anamosa	-	-	-	1	1
Hb Beograd	-	-	-	1	1

* There were 6 undefined hemoglobinopathies

During screening, some abnormal hemoglobins could not be defined. This feature was reported in molecular genetic analysis of β-thalassemia by Tadmouri et al. [21]. They suggested that samples can have a relatively high number of unidentified mutations, especially samples of individuals originating from southern and eastern Anatolia. They explained the possible presence of rare and novel mutations in these isolated areas where consanguineous marriages are practiced extensively. Our cases were not partners of hemoglobinopathy carriers but they were informed and a molecular genetic study was recommended.

The expected number of the infants born with β-thalassemia major annually is calculated to be 150-200 in Turkey. Approximately 800 pregnant women should seek prenatal diagnosis each year [22]. Screening for hemoglobinopathies is important even in regions with lower prevalence. Consanguineous marriages may increase high-risk couples. A cost-benefit analysis of prevention programs showed that the total cost of the program in preventing birth of a single child with thalassemia major was equal to or less than the cost of treating a patient for one year [23].

Follow-up of high-risk couples and acquisition of information about cancellation of the marriage, pregnancies and health of offspring were generally impossible due to unreliable phone numbers/addresses of the couples. A meaningful study evaluating the efficiency of premarital screening in Hatay showed that only 17.5% of pregnant high-risk couples underwent prenatal diagnosis despite genetic counseling [6]. These results show that education in the hemoglobinopathy prevention program is still inefficient. Especially couples with a low educational background can not fully comprehend the subject during premarital screening and genetic counseling. In a screening center in the Gaza Strip, a video about a thalassemia family is presented to carriers and explained by one of the staff, who is a thalassemia patient [24]. This method will be more effective in increasing the carrier's familiarity with the subject. Employing thalassemic patients in these centers also seems to be a good idea. Despite religious restriction in the community regarding

Table 3. Premarital screening studies from Turkey

Author/year	Province	Number of screened people	Prevalence of β–thalassemia carriers	Prevalence of sickle cell carriers
Kılınç M et al.* 1999	İçel	6746	3.1%	6.4%
Keskin A et al. 2000	Denizli	19804	2.6%	0.11%
Bolaman Z et al. 2001	Denizli	14200	2.2%	ND
Gali E et al. 2001	Hatay	10207	3%	8%
Güler E et al. 2007	Konya	72918	2%	0.05%
Acemoğlu H et al. 2007	Erzurum	1610	0.68%	0
Güler E et al. 2008	Kahramanmaraş	11040	2.3%	0.54%
Sarper N et al. 2009	Kocaeli	88888	0.89%	0.05%

*Also includes screenings in schools and community screening

abortion, free prenatal diagnosis must also be available. Relevant educational programs in schools and in the media are essential complements of any control program [25].

Hemoglobinopathy screening programs in addition to education programs should be improved and expanded throughout the country. The high morbidity of hemoglobinopathies, chronic transfusion requirements, high cost of chelating, organ damage, painful crisis and other crisis, and availability of stem cell transplantation for only a limited number of patients with compatible sibling donors justify premarital screening studies even in regions with lower prevalence such as Kocaeli.

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